

1/20/98

1/20 STNPR ELISA - Mouse Serum of C3H mice injected with
 • L929 cells or Clone 39 + Primary tumor cell lines
 of CL39.

- + Standard STNPR protocol
- + samples diluted in 0.1% BSA/PBS/Dulbecco
- + PNPP ~20min

	1	2	3	4	5	6	7	8	9	10	11	12	
A	CLK unirad 1:5	CLK #2 1:5	CLK unirad #3 1:5	CL39 unirad #1 1:5	CL39 unirad #2 1:5	CL39 unirad #3 1:5	CL39 #1-#5 Supt 1:50+	CL39 #3-#5 Supt 1:50+	CL39 #3-#5 Supt 1:50+	CL39 #3-#5 Supt 1:50+	CL39 #3-#5 Supt 1:50+	TNFBI 40 day/Ind	
B	1	2	3	4	5	6	7	8	9	10	11	12	
A A	0.736	0.620	0.137	0.344	0.136	0.335	0.533	0.584	0.570	0.478	0.484	0.553	
B B	0.179	0.184	0.140	0.257	0.146	0.207	0.526	0.474	0.540	0.398	0.388	0.456	
C C	0.186	0.187	0.157	0.164	0.200	0.142	0.136	0.124	0.115	0.084	0.117	0.157	
D D	0.754	0.718	0.193	0.759	0.779	0.710	0.177	0.148	0.177	0.135	0.450	0.477	
E E	0.187	0.145	0.264	0.744	0.164	0.131	0.201	0.156	0.140	0.099	0.458	0.503	
F F	0.207	0.170	0.306	0.765	0.200	0.144	0.514	0.183	0.195	0.076	0.071	0.443	
G G	0.750	0.757	0.340	0.758	0.142	0.079	0.146	0.540	0.521	0.435	0.537	0.606	
H H	0.144	0.136	0.218	0.318	0.203	0.118	0.179	0.545	0.499	0.430	0.437		
F	1:10	1:10	1:10	1:10	1:10	1:10	1:10	1:10	1:10	1:10	1:10	1:10	
G	CLK-S.D. #1 1:5	CLK-S.D. #2 1:5	CLK-S.D. #3 1:5	CL39-S.D. #1 1:5	CL39-S.D. #2 1:5	CL39-S.D. #3 1:5	CL39-S.D. #4 1:5	CL39-S.D. #5 1:5	CL39-S.D. #1 1:5	CL39-S.D. #2 1:5	CL39-S.D. #3 1:5	CL39-S.D. #4 1:5	CL39-S.D. #5 1:5
H	1:10	1:10	1:10	1:10	1:10	1:10	1:10	1:10	1:10	1:10	1:10	1:10	

mean A405 mouse Serum

	unirad	1.0 Gy	3.0 Gy	5.0 Gy
CLK 1:5	0.171	0.173	0.214	0.278
CLK 1:10	0.175	0.240	0.245	0.173
CL39 1:5	0.272	0.123	0.162	0.172
CL39 1:10	0.203	0.173	0.192	0.205

↳ Circulating
 STNPR being
 bound by
 circulating
 L-STNPR AI

mean A405 L929 Clone 39 Primary tumor cell lines from C3H mice:

$$\begin{aligned}
 1.0 \text{ Gy} - \#5 &= 0.801 & 5.0 \text{ Gy} \#1 &= 0.815 \\
 3.0 \text{ Gy} - \#1 &= 0.701 & 5.0 \text{ Gy} \#2 &= 0.760 \\
 3.0 \text{ Gy} - \#2 &= 0.620 & 5.0 \text{ Gy} \#3 &= 0.650 \\
 3.0 \text{ Gy} - \#5 &= 0.717 & 5.0 \text{ Gy} \#4 &= 0.701
 \end{aligned}$$

7/9/98

7/9 Determination of Total α -STNFR Ab in the Circulation
by mice immunized with denatured STNFR (See
pg 37 for info)

Groups of 5 mice each were immunized with denatured STNFR, PBS, or OEA alone and then challenged with LA29 clone 3G tumor cells. Blood was collected at time of necropsy. Samples were heated to 37°C for 10min, spun in microfuge for 10min at 3000 rpm, and the serum was collected.

Assay

- 1) Coat with 2ug/ml of goat α STNFR Ab - Block
- 2) Add 2ug/ml rhSTNFR
- 3) Add diluted mouse serum
- 4) Add 2ug/ml goat α mouse IgG, A, M - Phosphatase labelled
- 5) Add pNPP

	1	2	3	4	5	6	7	8	9	10
A	#1 1/400	#2	#3	#4	#5 → 1/25	PBS #4 1/25	1/50	1/100	NMS 1/400	NMS alone (no serum)
B	1/800				→ PBS 1/25	1/50	1/100	1/100		
C	1/1600				→ ADS #1	1/25	1/50	1/100	1/1600	
D	1/3200	X			→ ADS #2	1/25	1/50	1/100	1/3200	
E	1/6400				→ ADS #3	1/25	1/50	1/100	1/6400	
F	1/12800				→ ADS #4	1/25	1/50	1/100	1/12800	
G	PBS #1 1/25	1/50	1/100	PBS #3 1/25	1/50	PBS #5 1/25	1/50	1/100	2 ^o alone no serum	
H	PBS #2 1/25	1/50	1/100	1/100	1/3200	#2 NMS 1/25	1/50	1/100	STNFR alone (no serum)	

	1	2	3	4	5	6	7	8	9	10
A	0.1248	0.1201	0.1164	0.1144	0.1231	0.1274	0.1248	0.1274	0.1177	0.1110
B	0.1248	0.1204	0.1174	0.1144	0.1242	0.1287	0.1247	0.1288	0.1160	-----
C	0.1277	0.1245	0.1207	0.1187	0.1249	0.1322	0.1276	0.1324	0.1216	-----
D	0.1248	0.1204	0.1174	0.1144	0.1242	0.1287	0.1247	0.1288	0.1160	-----
E	0.1248	0.1204	0.1174	0.1144	0.1242	0.1287	0.1275	0.1295	0.1174	-----
F	0.1277	0.1245	0.1207	0.1187	0.1276	0.1322	0.1271	0.1324	0.1216	-----
G	0.1248	0.1204	0.1174	0.1144	0.1242	0.1287	0.1247	0.1288	0.1160	-----
H	0.1277	0.1245	0.1207	0.1187	0.1276	0.1322	0.1271	0.1324	0.1216	-----

9/4/98

9/4 - ID3 BI - Antibody Infusion + Challenge w/ Clone 39

These experiments are designed to evaluate the efficacy of ID3 BI (an α -SYNFR1 Ab) in protecting mice from Clone 39-derived L929 tumors. Mice will be injected with ID3 BI or Control Ab on day 0 (the time of tumor challenge) and again on days 3 and 6. Tumor development will be monitored daily.

- 9/4 (day 0) Inject ID3 BI or IBT.11 (λ -TNP Ab) IP - 0.5 mg Ab in ~ 1 ml per mouse
 ~ 11:00 AM using a 26 gauge needle (aliquotted using a pipetman, then inject all in ^{in media} _{10 sec})
 ~ 2:30 PM inject clone 39 Subcutaneously (mid-back) 10^7 Cells ^{in 5 ml} ₃₄₀
 26 gauge needle (aliquotted using a pipetman, then inject all)

9/7
 (day 3)
 Check mice for tumors - none at this time
 Inject mice with the appropriate antibody IP
0.5 ml containing 0.2 mg Ab in PBS

9/9
 (day 5)
 Check for tumors:
 ID3 BI injected mice = 4 tumors
 IBT.11 " " = 8 tumors

9/10
 (day 6)
 Check for tumors:
 ID3 BI = 8
 IBT.11 = 8

Inject mice with the appropriate Ab IP
 0.5 ml containing 0.2 mg Ab in PBS

* note: The IBT.11 mice actually got a total of 850 μ g of Ab - vs - 900 μ g of ID3 BI Ab mice.

9/11
 (day 7)
 ID3 BI = 8
 IBT.11 = 8

9/12
 (day 8)
 Same as above
 Resulted 2 tumors from IBT.11 group + 1 from ID3 BI group

11. tumors are regressing.

12. to optimize dosage, route of administration,
frequency of administration, etc.

13. end of Th. D. work - see Book #5 for Post-Doc
research

3/24/98

3/24 - STNFR ELISA of Purified ID3 and Clones of Limiting Dilution

- * Test fractions of ID3 purified from protein G column
- * Test 1st set of clones from limiting dilution

Procedure

- Coat 101 2ug/ml goat x hSTNFR
- Block
- Add AB supt (UG37 + phthalo/hSTNFR)
- Add appropriate dilut of test sample
- Add 2 ug/ml goat x mouse IgG, IgA, IgM - AP conjugate
- Develop as per usual
- PNPP incubated for ~5 hours at RT (the substrate was one of old stuff + some of new stuff - reaction was very poor)

	1	2	3	4	5	6	7	8	9	10	11
A	goat IgG neat	wash #1 neat	wash #2 neat	wash #3 neat	wash #4 neat	wash #5 neat	clone #2 2ug/ml	clone #3 2ug	clone #4 2ug	clone #5 2ug	clone #6 2ug
B	1/2	1/2	1/2	1/2	1/2	1/2	4ug	4ug	4ug	4ug/ml	4ug
C	1/4	1/4	1/4	1/4	1/4	1/4	sp20 Supt + Biotin alone	sp20 Supt alone	sp20 Supt alone (alone)	sp20 Supt alone (alone)	
D	4F2	4F2	4F2	4F2	4F2	4F2	1H7	3B11	3B11	3B11	F7
E	7F	7F	4F2 5D 10C	10C	B1	A12	1H7 (weak)	A2	B12	B8	
	2B8	1A8	1A8	1A8	1A8	1A8	1A8	1A8	1A8	1H7	4F2
	B12	B9	C2	D4	D5	E9	F10	F5	G8	D2	B6
	1	2	3	4	5	6	7	8	9	10	11
A	0.343	0.447	0.081	0.060	0.038	0.044	1.404	1.600	1.571	1.657	1.657
B	0.897	1.260	0.078	0.039	0.032	0.039	1.456	1.316	1.412	1.472	1.572
C	0.917	1.157	0.106	0.046	0.053	0.030	1.412	1.316	1.412	1.472	1.572
D	0.4126	0.4120	0.068	0.0133	0.065	0.060	0.041	0.041	0.041	0.037	0.032
E	0.085	0.075	5.778	0.1304	0.402	0.453	14.520	0.748	0.756	0.941E	

Patched: 4F2-7F, 4F, 10C and 1A8-C2, D4, D5, E9, F10, F5, G8
to 98 well: and 1H7-D2

ratio: purified Ab is biologically active 1/m in terms of binding to native STNFR). Concentration may be much lower than predicted.

3B11 may be a total bust - no positive clones and poor binding to 9B in previous assay (pg. 29)